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Possible Involvement of the Alternative Respiration System in the Ethylene-stimulated Germination of Cocklebur Seeds

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ABSTRACT

Respiration of nondormant upper cocklebur (*Xanthium pensylvanicum* Wallr.) seeds was enhanced by exogenous C_2H_4, proportionally to the concentration of C_2H_4 and the duration of presoaking of the seeds. Benzohydroxamic acid (BHM) and salicylhydroxamic acid (SHM), inhibitors of alternative respiration, inhibited both the germination of nondormant lower cocklebur seeds and the respiration of the upper seeds presoaked for periods of 12 to 30 hours. Both the growth and respiration of axial and cotyledonary tissues were also inhibited by BHM. Moreover, BHM inhibited both the C_2H_4-induced germination of the upper seeds and their C_2H_4-stimulated respiration; the inhibition occurred only with concomitant addition of C_2H_4 and BHM. The respiration of seeds with a secondary dormancy induced by presoaking for prolonged periods was markedly stimulated by C_2H_4 but not suppressed by BHM. It was suggested that the alternative respiration system may be involved in the normal germination process of cocklebur seeds, secondary dormancy may result from its inactivation, and C_2H_4 may exert its germination-promoting action by stimulating the alternative respiration. The effects of BHM and SHM can suggest but not prove the involvement of the alternative respiration in seed germination.

Ethylene is a plant hormone which plays a leading role in the regulation of cocklebur seed germination (2–4, 8, 9). C_2H_4 causes seed germination by stimulating the initial growth of both axial and cotyledonary tissues (3, 4); however, the mechanism by which C_2H_4 stimulates their growth is not known.

In a different system, C_2H_4 is capable of breaking the dormancy of potato tubers (12, 18), and it enhances their respiration (11, 13, 16). Similarly, the stimulative effect of C_2H_4 on the climacteric of fruit respiration is well known (10). Recently, these phenomena were indicated to be achieved through the activation by C_2H_4 of alternative, CN-resistant respiration (15–17). Yentur and Leopold (19) have suggested the involvement of the alternative respiratory system in seed germination. This is consistent with findings by Hendricks and Taylorson (7) that BHM[1] and acetohydroxamate, inhibitors of the alternative respiration, inhibited the germination of *Amaranthus* seeds. Thus, the C_2H_4 action in seed germination, through its promoting effect on the initial growth of seed tissues, may be associated with its stimulation of alternative respiration. The present study was aimed at testing this possibility using cocklebur seeds.

MATERIALS AND METHODS

Fully after-ripened, nondormant upper and lower cocklebur (*Xanthium pensylvanicum* Wallr.) seeds, were stored at 8 C since harvest and were used. The use of the lower seeds which were capable of germinating at 23 C was restricted to an experiment shown in Figure 1, in which germination tests were carried out in 9-cm Petri dishes with two discs of filter paper wetted with test solutions. The upper seeds, which were nondormant but incapable of producing enough thrust to overcome a mechanical restraint by their seed coat at 23 C (8), were used for C_2H_4-induced germination tests, measurement of seed tissue growth, and respiration assays. For germination tests, seeds were scattered over the surface of a double layer of filter paper moistened with 4-ml test solutions in 125-ml flasks. For growth assays, cotyledonary and 3-mm-long axial segments were separated by severance, immediately washed with water, blotted and weighed, and then arranged on a piece of filter paper (2 × 2 cm) in 30-ml vials containing 1-ml test solutions. Ethylene concentrations within the flasks and vials were prepared by adding the necessary volumes of the gas with a syringe through skirted rubber stoppers. C_2H_4-free flasks and vials for controls contained a small glass tube with 0.2 ml of 0.25 M $Hg(ClO_4)_2$ solutions as C_2H_4 absorbent. All data points are averages of triplicate flasks or vials, each of which contained 18 to 22 seeds or 12 segments. Growth of segments was determined by measuring their fresh weight at the end of treatments and is shown as the per cent increase over the original fresh weight.

O_2 uptake of 50 upper seeds, 50 axial and 30 cotyledonary segments was measured manometrically using a 30-ml vessel which was lined with a piece of filter paper wetted with H_2O or test solutions and equipped with two slender side arms and two small side vessels, the latter containing either CO_2 and C_2H_4 absorbents for C_2H_4-free controls, respectively, or CO_2 absorbent alone. For measurement of O_2 uptake as affected by C_2H_4 application, atmospheres within the flask were replaced with air containing the various concentrations of C_2H_4, by passing 400 ml C_2H_4-containing air through the two side arms and immediately sealing their entrance with stoppers. Before being arranged on filter paper seeds or seed segments were immersed in test solutions for 20 min.

All experiments were carried out at 23 C.

RESULTS

Inhibition of Germination and Respiration by BHM and SHM. In order to determine whether or not an alternative respiration system is involved in the germination and respiration of cocklebur seeds, germination (Fig. 1) and O_2 uptake (Fig. 2) in response to BHM and SHM, inhibitors of alternative respiration (14), were examined using unimbibed lower seeds and 16-h imbibed upper ones, respectively. Increasing BHM and SHM concentrations caused progressively increased inhibition of germination at 64 h after their application (Fig. 1). After 96 h of the treatments, the inhibitory effect of BHM still remained, but that of SHM disappeared. The disappearance of the SHM effect with time may be due to adaptive development of a SHM-destroying system. Also, respiration was increasingly inhibited by BHM or SHM as their concentrations were increased (Fig. 2). It was demonstrated that

[1] Abbreviations: BHM: benzohydroxamic acid; SHM: salicylhydroxamic acid.

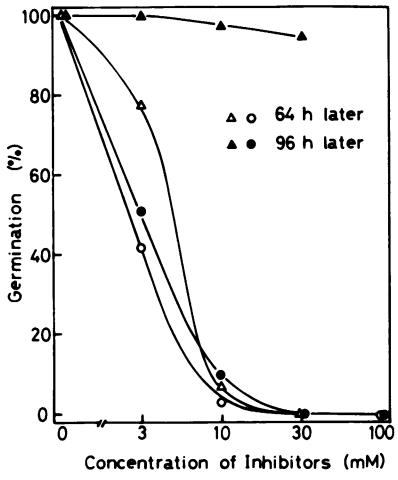

FIG. 1. Dose response curves of BHM and SHM in germination of lower cocklebur seeds. Data are shown by per cent germination after 64 and 96 h of treatments. (○, ●): BHM; (△, ▲): SHM.

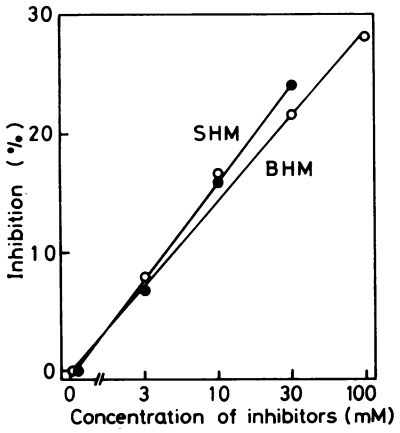

FIG. 2. Dose response curves of BHM and SHM in respiration of upper cocklebur seeds presoaked for 16 h. Data are shown by per cent inhibition of O_2 uptake rate in 4-h-treated seeds. Rate of O_2 uptake without inhibitors was 0.0293 μl/min·seed.

the alternative respiration may be involved in both the germination and respiration of cocklebur seeds.

However, an experiment in which 100 mM BHM was applied to the seeds which had been subjected to the different durations of water imbibition indicates that the alternative respiratory system is not involved in the earlier period of water imbibition, because the inhibition of O_2 uptake by BHM did not occur at the 5th h of water imbibition (Fig. 3). The respiration-inhibiting effect of BHM was pronounced only when it was applied during the period between the 15th and 30th h of presoaking, and again disappeared as the duration prior to BHM addition exceeded 3 days. Thus, the respiration of seeds with secondary dormancy was hardly suppressed by BHM.

Inhibition by BHM of Growth and Respiration of Seed Segments. The effects of BHM on the growth and respiration of axial and cotyledonary segments presoaked for 6 h prior to BHM treatment were examined (Figs. 4 and 5). Unlike KCN, which promoted both axial and cotyledonary growth (5), BHM strongly inhibited both over a wide range of concentrations; the axial tissue was more sensitive to BHM than the cotyledonary tissue. In both the axial and cotyledonary segments, BHM also caused inhibition of respiration in proportion to its concentration, the extent of the inhibition being greater in the axial tissue than in the cotyledonary tissue (Fig. 5). A 30% inhibition of respiration of the axial segments was elicited with about 30 times lower concentration of BHM than that required for inhibiting the respiration of intact seeds, perhaps suggesting low permeability to BHM of the cocklebur seed coat. The actual inhibitory effect of BHM was observed within 1 h of its application at a time when the axial growth had not been initiated. It is apparent that the inhibition of respiration by BHM cannot be the result of suppressed growth of seed tissues by BHM; however, there is a striking parallelism between dose response curves of BHM in the growth and respiration of both seed tissues.

Stimulation of Respiration by C_2H_4. The effects of a serial concentration of C_2H_4 on the respiration of upper cocklebur seeds presoaked for varying periods are shown in Figure 6. C_2H_4 application at the start of water imbibition had little effect on respiration, but in all cases increasing the concentration of C_2H_4 progressively stimulated the respiration of preimbibed seeds. The degree of stimulation increased with the duration of presoaking, being more than 2-fold in the secondarily dormant seeds presoaked for 2 months. The stimulatory effect of C_2H_4 on seed respiration was detected within 1 h of its application and reached a maximum at 4 h (Fig. 7). When treated with C_2H_4 at the time of water imbibition, in contrast, unimbibed seeds did not show any significant C_2H_4 stimulation of their respiration even 6 h after application of the gas (Fig. 7).

Inhibition of C_2H_4-induced Germination by BHM. Upper cocklebur seeds presoaked for 62 h were exposed to various concentrations of C_2H_4 in the absence or presence of 20 mM BHM. BHM

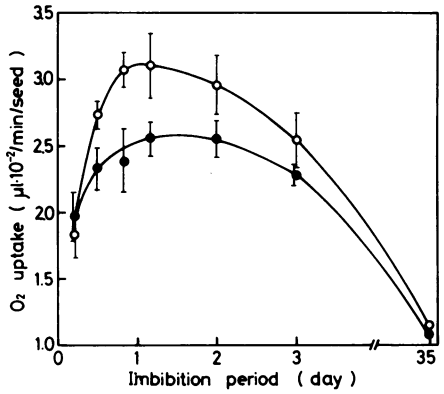

FIG. 3. Changes in respiration of upper cocklebur seeds during a water imbibition period and their respiration response to BHM. Seeds presoaked for varying periods were contacted with (●) or without (○) 100 mM BHM and data after 5 h were plotted in the figure with standard deviation calculated from three to five replicates.

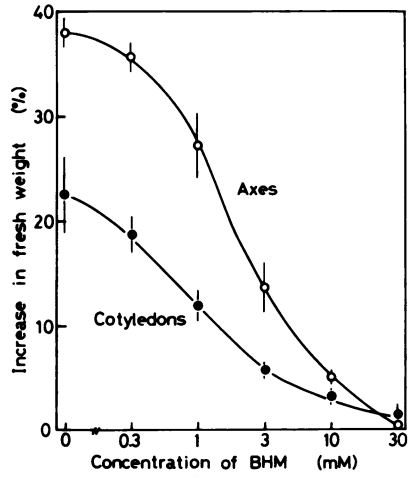

FIG. 4. Dose response curves of BHM in growth of axial and cotyledonary segments excised from upper cocklebur seeds. Segments were presoaked for 10 h. Data for axial and cotyledonary growth were taken after 17 and 44 h, respectively.

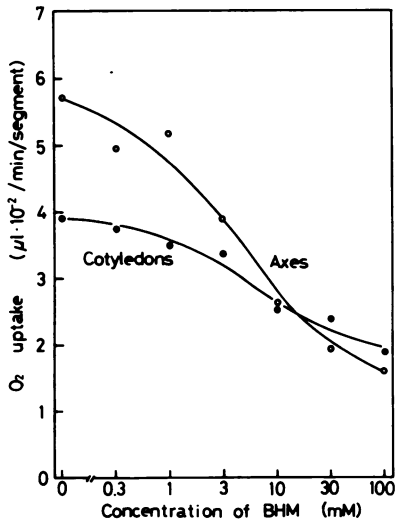

FIG. 5. Dose response curves of BHM in respiration of axial and cotyledonary segments excised from upper cocklebur seeds. Segments were presoaked for 10 h. Data are shown at O_2 uptake rate after 5 h of treatment.

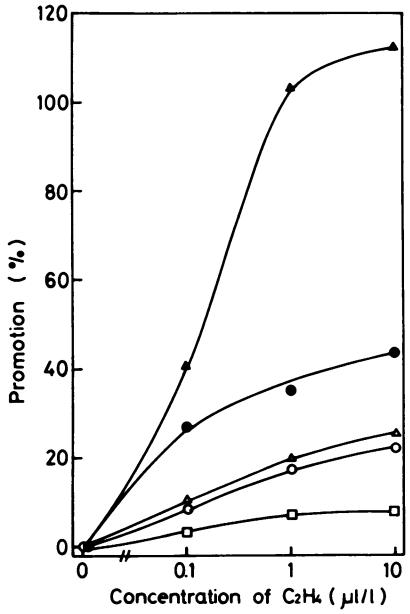

FIG. 6. Dose response curves of C_2H_4 in respiration of upper cocklebur seeds presoaked for different periods. Data are shown by per cent promotion of O_2 uptake rate by C_2H_4 in 4 h-treated seeds. (□): unimbibed; (○): 12-h imbibed; (△): 35-h imbibed; (●): 16-days imbibed; (▲): 60-days imbibed.

completely inhibited the C_2H_4-induced germination (Fig. 8) suggesting the involvement of the alternative respiration system in the C_2H_4 action.

However, the data in Table I show that BHM can act only when given together with C_2H_4. BHM addition prior or subsequent to C_2H_4 application did not show any inhibitory action on the C_2H_4 stimulation of seed germination.

Inhibition of C_2H_4-stimulated Respiration by BHM. The results in Table I suggest that respiration enhancement by C_2H_4 may be due to an increased activity of an alternative respiration system. Hence, the effectiveness of 100 mM BHM on respiration was compared between treatments with or without 10 μl/liter C_2H_4 using the upper cocklebur seeds presoaked for different periods (Table II).

In all cases, the inhibitory effect of BHM was more pronounced with its concomitant application with C_2H_4 than with its application alone. In the absence of applied C_2H_4, the effectiveness of BHM decreased as the seeds aged on water substratum and was completely lost at the secondarily dormant state (Fig. 3). In the presence of C_2H_4, in contrast, the effectiveness of BHM increased with aging of the seeds. O_2 uptake in secondarily dormant seeds was hardly stimulated by C_2H_4 combined with BHM. It is thus likely that the enhancement of seed respiration by C_2H_4 may result largely from the activation of the alternative respiration by C_2H_4.

Inhibition by BHM of C_2H_4-stimulated Growth in Seed Tissues. Axial and cotyledonary segments presoaked for 6 h were incubated with or without 3 μl/liter C_2H_4 with varying concentrations of BHM. Table III shows that the stimulatory action of C_2H_4 on axial and cotyledonary growth was decreased by increasing BHM concentration. Growth stimulation by C_2H_4 was hardly recognized in the presence of 10 mM BHM. These results indicate that the failure of the C_2H_4-induced seed germination in the presence of BHM is due to the inability of its seed tissues to grow in response to C_2H_4.

DISCUSSION

From the findings that KCN and NaN_3 can induce the germination of cocklebur seeds even on their way to secondary dormancy, it has been suggested that a CN-insensitive, alternative respiration system may be implicated in the germination process of this seed (5). As shown in Figures 1 and 2, BHM and SHM,

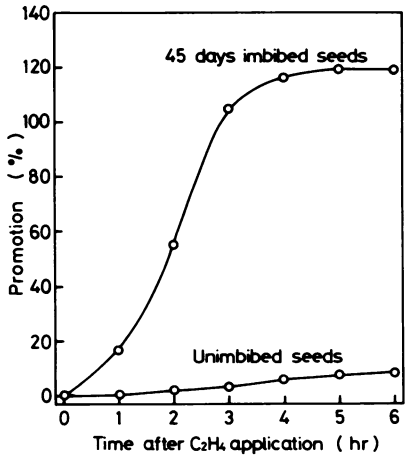

FIG. 7. Promotion by C_2H_4 of respiration in seeds of different ages. Upper seeds unimbibed or imbibed for 45 days prior to C_2H_4 application were exposed to 10 μl/l C_2H_4. Each spot is shown by per cent of O_2 uptake of C_2H_4-treated seeds to that of C_2H_4-free controls.

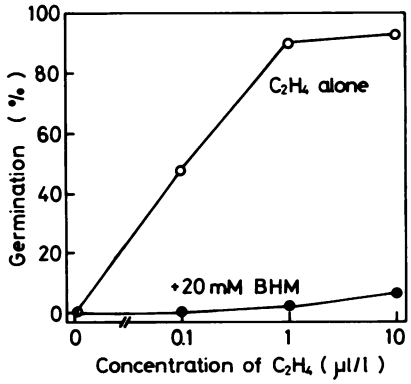

FIG. 8. Suppression by BHM of germination-inducing effect of C_2H_4. Upper cocklebur seeds presoaked for 62 h were exposed to various concentrations of C_2H_4 in the presence or absence of 20 mM BHM and after 5 days the numbers of germinants were counted.

Table I. Interaction of BHM and C_2H_4 in the Regulation of Cocklebur Seed Germination

According to the legends to Table, 72 h pre-soaked upper cocklebur seed were treated by variously combined 30 mM BHM and/or 20 µl/l C_2H_4, then transferred to a condition for germination test, and 3 days later the numbers of germinants were counted.

Treatment during		Germination (%)
First 8 hr	Second 8 hr	
air, H_2O	air, H_2O	0
air, H_2O	air, BHM	0
air, H_2O	C_2H_4, H_2O	20.4
air, BHM	C_2H_4, H_2O	17.5
C_2H_4, H_2O	air, BHM	22.2
air, H_2O	C_2H_4, BHM	1.9

Table II. Inhibition of BHM of C_2H_4-Stimulated Respiration in Upper Cocklebur Seeds Pre-Soaked for Different Periods

Data were taken after 5 h of C_2H_4 application. Each value represents the mean ± standard deviation from 3 to 6 replicates and numerals in parentheses show percent of the controls.

Duration of pre-soaking (day)	O_2 uptake (10^{-2} µl/min/seed)			
	Air		10 µl/l C_2H_4	
	H_2O	100 mM BHM	H_2O	100 mM BHM
2	2.87 ± 0.19	2.35 ± 0.15 (81.9 %)	3.40 ± 0.24	2.52 ± 0.20 (74.1 %)
9	1.42 ± 0.10	1.36 ± 0.11 (95.8 %)	2.62 ± 0.18	1.86 ± 0.12 (71.0 %)
65	0.72 ± 0.05	0.88 ± 0.07 (122.2 %)	2.24 ± 0.14	1.07 ± 0.08 (47.8 %)

inhibitors of alternative respiration (14), inhibited the germination and respiration of cocklebur seeds. BHM more strongly inhibited both the axial and cotyledonary growth (Fig. 4), as well as the O_2 uptake of the axial and cotyledonary segments (Fig. 5). It is thus likely that the alternative respiration system may be involved in some way in the germination process of cocklebur seeds. However, it is also possible that the effect of BHM and SHM on germination was achieved by a mechanism separate from their modification of respiration. It will be necessary to seek other evidence to confirm the role of alternative respiration in seed germination suggested by this study.

In contrast to the case of soybean seeds in which the respiration system shifted from the CN-insensitive type to the CN-sensitive one during an early period of water imbibition (19), the inhibition of O_2 uptake in cocklebur seeds by BHM was found only after 12 h of water imbibition (Fig. 3). This suggests that the suspected alternative respiration system in cocklebur seeds may be activated during water imbibition.

Ethylene stimulated not only the germination of cocklebur seeds but also their respiration in response to its increased concentrations (Fig. 6). Such stimulation of respiration by C_2H_4 occurred prior to the protrusion of a radicle or cotyledonary end during germination, and was observed within 1 h of its application (Fig. 7). These facts suggest that the induction of seed germination by C_2H_4 may be associated with the increase of respiration by C_2H_4.

The germination-inducing effect of C_2H_4, like the action of BHM shown in Figure 3, did not occur during an early period of water imbibition (9). Similarly, the respiration-stimulating effect of C_2H_4 was less striking soon after water imbibition (Fig. 6). Thus, one might suggest that such a C_2H_4 effect did not occur until the alternative respiration system developed. The germination-inducing and respiration-stimulating effects of C_2H_4 were

Table III. Inhibition by BHM of C_2H_4-Stimulated Growth of Axial and Cotyledonary Segments

Segments were pre-soaked for 6 h. Data for axial and cotyledonary growth were taken after 17 and 44 h, respectively, and shown by percent increase in fresh weight.

Tissue	Addition	Concentration of BHM (mM)				
		0	0.3	1	3	10
Axis	$Hg(ClO_4)_2$	29.9 ± 1.4	24.7 ± 1.6	23.2 ± 3.1	10.1 ± 2.4	4.2 ± 2.2
	3 µl/l C_2H_4	54.0 ± 0.5	40.4 ± 2.4	32.4 ± 2.0	13.5 ± 0.7	3.9 ± 0.9
	% promotion	80.6	63.6	39.7	33.7	-7.2
Cotyledon	$Hg(ClO_4)_2$	21.9 ± 1.8	19.7 ± 3.1	11.9 ± 1.5	5.8 ± 1.1	3.5 ± 2.8
	3 µl/l C_2H_4	33.9 ± 2.9	28.0 ± 1.8	16.4 ± 2.7	7.5 ± 1.2	3.8 ± 0.7
	% promotion	54.8	42.1	37.8	29.3	8.8

both insignificant in the presence of BHM; BHM strongly inhibited these C_2H_4 actions (Fig. 8 and Table II). Also, the C_2H_4 stimulation of the axial and cotyledonary growth disappeared in the presence of BHM (Table III). These facts would suggest that C_2H_4 exerts its germination-inducing effect through the activation of an alternative respiration system similar to systems in potato tubers (16) and some fruits (15, 17).

The suppression of the C_2H_4 action by BHM occurred only with its concomitant addition with BHM, and BHM failed to inhibit C_2H_4 action when given either preceding or following C_2H_4 addition (Table I). This may indicate that although the alternative respiration system is probably essential for the germination of cocklebur seeds, this system is not involved in the growth process of the axial and cotyledonary tissues subsequent to the certain step which is activated by C_2H_4. The action of C_2H_4 would be only to activate the alternative respiration system, which would lead to the increased axial and cotyledonary growth and consequently to the occurrence of seed germination.

The respiratory capacity of cocklebur seeds declined gradually after reaching a maximum at 24 h of water imbibition (Fig. 3). This change was accompanied by a decreasing sensitivity to BHM, and when the seed had entered into secondary dormancy, BHM could no longer exert its inhibitory effect on respiration (Fig. 3). At that time, not only the respiration-stimulating effect of C_2H_4 (Fig. 6) but also the inhibiting action of BHM against the C_2H_4-stimulated respiration (Table II) were highly pronounced. These facts may suggest that the secondary dormancy results from the inactivation of the alternative respiration system existing latently in the seed, and that C_2H_4 can perhaps reactivate the inactivated one, thus leading to the germination of seeds which are on the way to the secondary dormancy. Since the ability of seeds such as cocklebur (6) and lettuce (1) to produce C_2H_4 becomes small as they enter secondary dormancy, the inactivation of the alternative respiration system in the process of entry into secondary dormancy may be due to the lowered C_2H_4 level within the secondarily dormant seeds.

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